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Development of a Synthesis Method for O2-releasing Compound for Microbiological Experiments

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Abstract

Many celestial bodies within our solar system may have habitable environments due to the presence of liquid water. Europa, one of Jupiter's moons, may be habitable because of its liquid ocean and other potentially biologically favorable conditions. The ocean on Europa is hypothesized to contain large amounts of oxidants and low pH due to the radiolytically processed icy ocean shell. This suspected environment on Europa is similar to the composition of acid mine drainage on Earth, which can house microbial communities in environments of extreme acidity. Similar chemical reactions in Europa's ocean may occur to produce the appropriate reduction-oxidation gradients to support a small biomass. On Europa, the reactive oxygen species, could react with dissolved Fe(II) in the ocean, which creates Fe(III) oxides that can be reduced by the sediment on the ocean floor. This reaction could produce sufficient energy to support life if oxidants can be delivered to reducing sediments. This research aims to create oxygen slow releasing materials to mimic an iron snow reaction in an environment that is hypothesized to be similar to Europa's ocean composition. To do this, a synthetic acid mine drainage (SAMD) solution was created to mimic the hypothesized environment of Europa's ocean and different oxygen slow releasing treatments were performed to examine the rate of Fe(II) oxidation. The results suggested that the prepared oxygen slow releasing materials did oxidize Fe(II). The oxygen releasing compounds used in this study have important implications for future research on reduction-oxidation gradients in environments similar to Europa's ocean.

Introduction

It has been discovered that celestial bodies within our solar system may contain oceans of liquid water. Europa, one of Jupiter's four moons, has a liquid ocean, and has potential habitability due to its ocean, ice shell, atmospheric composition, and geology. The Voyager and Galileo missions revealed that the surface of Europa is younger than Earth and has water ice (Howell & Pappalardo, 2020). These conditions could support life because they can create physiochemical conditions favorable for stable biological structures and biologically essential elements. Due to the lack of sunlight, it is unlikely that life is driven by photosynthesis on Europa. Because of this, chemical energy would be needed to support life (Hand et al., 2009). The surface of Europa receives radiation from high energy charged particles from Jupiter's magnetosphere, which could create reduction-oxidation (redox) gradients as a source for chemotrophic life. This could create a habitable environment when the icy shell is radiolytically processed, causing H_2O molecules to alter to oxygen molecules, peroxide, and other oxidized compounds.

$$2\ H_2O \rightarrow 2\ H_2 + O_2$$

If these oxidized compounds can properly be introduced into the liquid ocean underlying the icy shell of Europa, they can increase potential for life on Europa (Hand et al., 2009). Although these oxidants can be harmful to life, there is indirect evidence suggesting that volcanism and formation of mafic and ultramafic magmas could yield energy when coupled with oxidants from the radiolytically processed icy shell with the reducing environment provided by water-rock interactions on the ocean floor (Papalardo et al., 2009). Considering the suspected environment of Europa, organisms would have to endure an abundance of oxidants and low pH due to a large amount of oxidants reacting with compounds and creating acids. This type of environment is similar to that of acid mine drainage, which can host microbial communities, thus suggesting that life is possible on Europa if it possesses the implicated environments (Pasek and Greenberg 2012).

Europa, like other moons and planets, is thought to be primarily composed of mafic silicate rocks. This is supported by Galileo radio-tracking gravity data that demonstrates differentiated internal structure, a dense core of metal or metal sulfides, a rocky mantle, and an ice-crusted ocean. The ice-crusted ocean is estimated to be 80-170 kilometers thick (Kargel et al., 2000). Doppler gravity data from the Galileo flybys suggest that along with this outer layer of H_2O, there is an anhydrous silicate mantle covering a metallic core (Papalardo et al., 2009). Along with the saltwater liquid ocean, there are geologic processes that can be inferred such as eruptions of icy liquids, widespread upwelling, fracturing of the ice crust as a result of tidal disruption, extension of lithospheric blocks, and upwelling that fills the voids left from the extension of lithospheric blocks (Kargel et al., 2000). Also, dark spots, domes, and chaotic terrain are present on the surface of Europa, suggesting tidally driven convection (Papalardo, 2010).

The surface of Europa is thought to be geologically young. Of the 75% of the surface that has been observed, only 23 impact features exceed 10 km in diameter. When this information is compared to models of recent impactors, it is estimated that Europa is approximately 40-90 Myr (Papalardo, 2010). Europa’s young age is supported because it has fewer craters than all other solid objects in the solar system. This is because cratering rates have dramatically decreased within the last 3.5 billion years, which may explain the lack of craters on the surface (Papalardo et al., 2009). Because of the surface’s youth, if a subsurface ocean was present during the formation of the satellite, the ocean should exist (Papalardo, 2010). During the Galileo flybys that occurred during 1996-2000, doppler gravity data revealed the surface of Europa is layered. This data indicated a rocky mantle and an iron core covered by an H_2O rich layer. The doppler data that the Galileo flybys provided could not indicate if the H_2O layer consisted of liquid water, but Galileo magnetometer data provided stronger evidence for liquid water on Europa because of an induced magnetic field which may suggest current or past conditions of a liquid ocean (Papalardo et al., 2009). Some impact structures, described as "multi-ring structures," also indicate a subsurface ocean. These structures have concentric rings and suggest an impact through the icy shell into the liquid ocean, which may explain their uniqueness (Papalardo, 2010). It is suspected that Europa is geologically active, and there is speculation that there is possibly resurfacing and high regional heat flow. Despite this, it is clear that Europa has a geologically young surface and a geologic history signifying oceanic influences (Kargel et al., 2000). While a subsurface ocean increases the likelihood of life being able to survive on Europa, many conditions, such as sufficient heat would be needed for favorable conditions. Fortunately, there is evidence that there may be sufficient heating on Europa.

Because many satellites like Europa lose heat that would melt oceans over geological time, it was thought that Europa would be frozen solid today. Presently, calculations reveal that there may be tidal heating that may allow Europa's icy shell to maintain a liquid ocean (Papalardo, 2010). Features on Europa, such as chaos terrain and lenticulae, may suggest melting and disruption of the icy surface, despite the cold temperatures on the surface of Europa. The chaos and lenticulae may be caused by rising diapirs, which would create melting of the icy shell. These diapirs are heated up by tidal forces, which can be inferred from laboratory experiments according to Sotin et al., (2002). When this tidal energy is concentrated on rising plumes, the plumes will reach the base of the icy shell, and spread laterally, causing the chaos, lenticulae, and partial melting. Through three different laboratory models, Sotin et al., (2002), propose that melting and the surface structures can occur, even if the ice layer is larger than 20 km (Sotin et al., 2002). These models show that because there is thought to be a liquid ocean caused by partial melt, there may be sufficient heating on Europa. Data also shows an induced magnetic field that indicates the presence of plumes. These plumes may release water into space, suggesting shallow reservoirs of water beneath the surface of Europa (Howell & Papalardo, 2020). The E26 flyby in 2000 indicated that there is a subsurface electrical conductor planet-wide on Europa, with water being the most probable substance. The Galileo magnetometer measurements also support that the geologic features are due to a subsurface ocean (Kilvelson et al., 2000). Not only did the magnetometer provide evidence for the liquid ocean, but it also showed that the H_2O layer was relatively thin. Because of the thinness of the H_2O layer, pressures would not allow ice to form below the surface of the ocean, which would cause the ocean to be in direct contact with the rocky mantle (Howell & Papalardo, 2020).

To support life, liquid water, energy sources, physiochemical conditions favorable for stable biological structures, and biologically essential elements would be needed (Hand et al., 2009). Since phototrophic life is unlikely on Europa, chemotrophic life would need energy in the form of redox gradients to be able to support life. Heat is needed to create sufficient cycling of these oxidants and reductants needed to support a biomass. The orbital resonance between Io, Europa, and Ganymede may cause increases in heat fluxes in the mantle due to the gravitational influence the celestial bodies have on each other. If tidal heat is produced in the mantle of Europa, hydrothermal processes would be able to take place (Zolotov & Shock, 2004). Because of the tidal heat, hydrothermal vents could cycle the oxidants at the surface (Marion et al., 2003). These hydrothermal systems may be similar to those on Earth’s ocean floor. On Earth, there is microbiological activity near the hydrothermal systems and because of this, hydrothermal systems may be able to support similar organisms (McCollom, 1999). Also, thermal fluxes in cryovolcanism could also support cycling of nutrients (Marion et al., 2003). Evidence of cryovolcanism, such as plumes of water, has been identified from observations on the Hubble Space Telescope. This evidence may suggest that these eruptions of water may be consistently active on Europa, supported by thermal anomalies at the same location. Also, this evidence is seen to be geologically recent, supporting the hypothesis that cryovolcanism may still be occurring today. (Sparks et al., 2017). If there is sufficient cycling between the icy shell and the ocean floor due to hydrothermal activity and cryovolcanism, conditions would be favorable for a small biomass because of the reduction-oxidation gradients that are created (Marion et al., 2003).

As a result of the ocean-mantle contact, nutrients that could potentially support life would be easily supplied to Europa's ocean and cycled due to the tidal heat and cryovolcanism. Photosynthesis is an unlikely mechanism for life, so chemosynthetic life may exist, and there is sufficient evidence to suggest that chemical disequilibrium exists in Europa's ocean (Papalardo, 2010). Firstly, due to the heavy radiation levels, Europa's surface is very oxidizing (Marion et al., 2003). When the radiation reaches Europa's surface, some H_2O molecules are transformed into oxidized compounds such as oxygen (O_2) and peroxide (H_2O_2) (Papalardo, 2010). These high amounts of strong oxidants at the surface of Europa will likely cause the surface to be sterile. Because of this, if life is present on Europa, it would most likely be in the subsurface, Also, liquid water and nutrients will be more readily available in the subsurface (Zolotov & Shock, 2004). Considering the inferred thickness of the icy shell, convection is a likely mechanism to supply the oxidants to the liquid ocean (Papalardo, 2010). Because of this, oxidant availability might not limit abiotic or biological reactions and suggests the possibility that the ocean is capable of supporting biologic activity that may range from single celled microbes to complex multicellular life forms (Hand et al., 2009). Presumably, the ocean and the ocean floor are reducing environments. Because of the lack of direct observation of reductants, laboratory simulations are used to infer the reductant availability on Europa (Hand et al., 2009). It is widely accepted that Europa has a rocky interior under the subsurface ocean. Volcanism and submarine hydrothermal systems may be present because of the friction from tidal forces, which heats Europa's interior (McCollom 1999). Igneous rocks that are exposed at the ocean floor may contain minerals such as FeS, magnetite, and silicates (Zolotov & Shock, 2004). Due to these hydrothermal systems reacting with the liquid ocean, the ocean seafloor of Europa is thought to be a reducing environment (McCollom 1999). Reductants such as CH_4, H_2, H_2S, and Fe^{2+} are thought to be hydrothermally derived on the seafloor of Europa (Hand et al., 2009). This chemical disequilibrium between the oxidants in the surface and reductants on the seafloor could produce enough energy for microorganisms. These conditions can be analogous to some anoxic environments for microorganisms in the continental subsurface. In these anoxic conditions, the electron acceptors are ferric iron, nitrate, nitrite, sulfate, CO_2, native sulfur, and MnO_2, where the electron donors would be H_2, methane, organic compounds, sulfides, native sulfur, ferrous iron, and $Mn(II)_{+}$ (Zolotov & Shock, 2004).

Since it is thought there is an abundance of oxidants formed at the surface of Europa, the subsurface ocean is assumed to be acidic. The low pH of Europa is due to the large number of oxidants reacting with sulfides. These reactions would cause sulfuric acid to be formed. As mentioned above, there would be sufficient amounts of oxidants to support significant life. However, the large amounts of oxidants suspected would likely cause oxidation and acidification of the ocean, which would cause the subsurface to have a pH of approximately 2.6. Organisms that could survive this environment would have to endure a large quantity of oxidants and low pH (Pasek & Greenberg, 2012). An analogous environment to these suspected conditions is areas that are affected by acid mine drainage. Low pH from acid mine drainage is often caused by oxidation of sulfide minerals, which is similar to the suspected environment on Europa (Marion et al., 2003). In these environments, there are communities of microorganisms that are able to survive this extreme acidity (Edwards et al., 2000). These organisms oxidize iron and sulfides as a source of energy, so Fe^{3+} could be an efficient oxidant, which would cause the environment to remain acidic (Pasek & Greenberg, 2012). An example of this is a species of Archaea that is found in Iron Mountain, California. This particular species survives in conditions with pH of 0 (Edwards et al., 2000). This is the lowest pH ever recorded in a natural system and provides an analogous environment to the suspected conditions on Europa. While the acidity of the ocean may limit the possibility of life on Europa, Iron Mountain, and other acidic environments on Earth show that life can adapt to extreme environments (Marion et al., 2003). Also, if sulfide is the dominant reductant on Europa, the reactions seen in AMD ecosystems would be possible in Europa's ocean (Pasek & Greenberg, 2012).

In order to support life, Europa's ocean would need to produce redox gradients, since all known life on Earth requires these potentials to obtain energy from the environment to exchange for cellular energy, which enables cellular maintenance, metabolism, and reproduction (Papalardo et al., 2009). The objective of this research is to conduct important preliminary work to better understand the potential for life on Europa. This research will study how to synthesize oxygen releasing compounds (ORCs) to mimic the radiolysis of the icy shell on Europa. These ORCs will help future experiments concerning redox gradients on Europa. To do this, a model was created to deliver oxidants to reducing sediments. On Europa, the reactive oxygen species, react with dissolved Fe(II) in the ocean. Once the Fe(III) oxides settle to sediments, they are then reduced which could create energy to support a small biomass. This experiment will examine the rates of Fe(II) oxidation in these conditions to know how to manipulate the rates of this oxidation, so further studies can examine how bacteria in synthetic AMD (SAMD) environments respond to different rates of oxidant delivery to see if these conditions would be suitable for life in Europa's ocean. This experiment will create oxygen-generating materials to mimic iron snow, and using the reactive oxygen species and Fe(II) reaction to mimic Europa's ocean. These reactions will allow us to manipulate the rates of oxidation to better understand how to join Fe(III) and sediments on Europa for future experiments. The goal of this study is to create a system to mimic the hypothesized environment of Europa's ocean, prepare oxygen releasing pellets, and test to see how fast Fe(II) oxidizes in SAMD.

Materials and Methods

All reagents were purchased from VWR. A SAMD solution was created based on the solution used in Senko et al. (2008). This solution consisted of 450 mL of ultrapure water (resistivity > 18bMΩ.cm), 5 mM $CaSO_4$, 4 mM $MgSO_4$, 1 mM Na_2SO_4, 0.5 mM $Al_2(SO_4)_3$, 0.4 mM $MnSO_4$ and 0.1 mM $(NH_4)_2 Fe(SO_4)_2$. The solution was then adjusted to a pH of 4.0 with 1 M H_2SO_4 and then final volume was brought up to 500 mL with ultrapure water. The solution was then bubbled with nitrogen for 45 minutes, sealed with a rubber stopper, and then brought into a Coy Laboratory Products glove bag. A stock solution of 100 mM $FeSO_4$ was also created and also brought into the glove bag.

Oxygen slow-releasing materials (OSRMs) were created based on OSRMs created by Zhou et al., (2017). In this study, there were two different OSRMs created: one with stearic acid and one without. Both OSRMs consisted of CaO and quartz sand with a ratio of 42:13. The OSRM with stearic acid had a ratio of 1:42:13 of stearic acid to CaO to quartz sand. Both the no-SA OSRM and the SAOSRM were added to 100 mL of methanol and stirred until a slurry was created. Once stirred, the no-SA OSRM and the SAOSRM were baked overnight at 80 °C. The OSRMs were stored at room temperature.

Each incubation consisted of 50 mL of SAMD solution and 1 gram of an oxygen slow releasing compound in two of the incubations. There were three incubations: a control with no oxygen slow releasing compound, oxygen slow releasing compounds without stearic acid, and an oxygen slow releasing compound with stearic acid. All the incubations were prepared in triplicate and stored in a glove bag. Each bottle was injected with 5 mL of 100 mM $FeSO_4$ stock solution and was gently swirled. Immediately, 0.1 mL was extracted and added to 0.1 mL of HCl in a microcentrifuge tube to measure the total Fe(II). Also, 0.2 mL was added to an empty microcentrifuge tube to measure the soluble Fe(II). Each tube was centrifuged in an Eppendorf Minispin centrifuge for five minutes at maximum speed of 13.4 revolutions per minute. Once centrifuged, 0.1 mL of the supernatant was extracted with an Eppendorf Research pipette from the total Fe(II) and put into an empty microcentrifuge tube and 0.1 mL of the soluble Fe(II) was pulled and put into a microcentrifuge tube that contained 0.1 mL of HCl. The HCl preserved the samples until they were measured for the Fe(II) concentrations. Samples were collected again after two hours of the initial preparation. The final sample was collected after 35 days.

Fe(II) concentrations were determined for both the total and soluble iron by ferrozine assays (Stookey, 1970). A Thermo Scientific Genesys 20 spectrophotometer was used to determine the Fe(II) content at 562 nm. For each sample set, HCL was used to zero the spectrometer, and Fe(II) standards at 0.1 mM, 0.25 mM, 0.5 mM, and 1 mM were performed.

Results

To determine the rate of Fe(II) oxidation by the reactive oxygen species, the concentrations of soluble Fe(II) and total Fe(II) were measured at t=0 days and t=35 days (Figures 1 & 2). Soluble concentrations are lower than total Fe(II) concentrations because the soluble concentration is only what is dissolved in the solution. The total Fe(II) is the dissolved and absorbed Fe(II). These incubations were prepared in triplicate, so the soluble and total Fe(II) concentration are an average of the three concentrations of each treatment.

For this experiment there were three treatment types: SAOSRM, no-SA OSRM, and no OSRM. These treatments were performed to release oxygen in the SAMD conditions to induce Fe(II) oxidation that would occur in an environment similar to Europa's ocean. These treatments were based on the ORCs created by Zhou et al., (2017). CaO was used in both treatments because it can release oxygen when it reacts with water. This reaction causes the oxygen from the reaction to become an electron acceptor (Zhou et al., 2017). This allows the reaction of Fe(II) losing an electron to get the Fe(III) oxides that would be reduced to create energy for a small biomass in Europa's ocean. The SAORC treatment included stearic acid to slow down the release of oxygen. The stearic acid slows down the release of oxygen because it is hydrophobic and will embed some of the CaO under anhydrous conditions (Zhou et al., 2017).

For the soluble Fe(II) concentrations, there was a decrease in Fe(II) concentrations from t=0 days to t=35 days. This is partly due to the fact that there are lower concentrations because the dissolved Fe(II) was only measured and the dissolved and absorbed Fe(II) was measured in the total Fe(II) concentrations. For the SAOSRM, the soluble concentration was initially 0.077

mM and had a lower concentration of 0.051 at t=35 days. For the no-SA OSRM, the initial soluble concentration was 0.038 mM at t=0 days and 0.012 mM at t=35 days. The control group, the OSRM-free incubations, had a soluble Fe(II) concentration of 3.9 mM at t=0 days and 3.9 mM at t=35 days. This is an important control because it helps show the extent of how the OSRMs affected the soluble Fe(II) concentrations (Figure 1). The OSRMs affected the soluble Fe(II) concentrations immediately and caused the soluble Fe(II) concentration to decrease compared to the initial soluble Fe(II) concentration with no OSRMs. This decrease may be due to the Fe(II) being absorbed because of the addition of OSRMs. Because this dissolved Fe(II) was not detected, it cannot be determined if oxidation occurred until the total Fe(II) concentrations are examined.

For the total Fe(II) concentrations, there was also lower Fe(II) concentration at t=35 days compared to t=0 days. The SAOSRM had a beginning total Fe(II) concentration of 3.8 mM at t=0 days and a concentration of 1.5 mM at t=35 days. The no-SA OSRM had a total Fe(II) concentration of 4.1 mM at t=0 days and 2.1 mM at t=35 days. Lastly, the treatment with no OSRMs had a beginning toral Fe(II) concentration of 3.9 mM at t=0 days and a concentration of 2.9 mM at t=35 days (Figure 2). Unlike the soluble Fe(II) concentrations, the OSRMs did not have an effect on the total Fe(II) concentration at t=0 days because the total Fe(II) concentration account for both the dissolved and absorbed Fe(II).

The purpose of this study was to create oxygen-generating materials to mimic iron snow, and using the reactive oxygen species and Fe(II) reaction to mimic Europa’s ocean. This model examined the rates of Fe(II) oxidation in an environment similar to Europa’s ocean to help determine how to manipulate these oxidation rates to better understand how to join Fe(III) and reducing sediments for future experiments on the habitability of Europa. Because there was a lower Fe(II) concentration for the two oxygen releasing compound treatments for both the soluble Fe(II) and the total Fe(II) at t=35 days, it can be determined that there was Fe(II) oxidation from the OSRMs. The treatments with no OSRMs for the soluble Fe(II) did not decrease and had a much larger beginning and ending concentration, which demonstrated that the OSRMs worked on the soluble concentrations (Figure 1). For the total Fe(II) concentrations with no ORSMs, there was a decrease in the Fe(II) concentrations from t=0 days to t=35 days, but this decrease was much less than the decrease seen in the SAOSRM and the no-SA OSRMs (Figure 2). The effect of the stearic acid in the OSRMs had only a slight effect on the Fe(II) concentrations. For the soluble Fe(II) concentrations, the SAOSRM had a beginning concentration of 0.077 mM and a concentration of 0.051 at t=35 days. For the soluble concentration for the no-SA OSRM the beginning concentration was 0.038 mM at t=0 days and 0.012 mM at t=35 days. This is expected because the stearic acid was used to slow oxidation rates (Zhou et al., 2017). For the total Fe(II) concentrations, the SAORSM had a beginning concentration of concentration of 3.8 mM at t=0 days and a concentration of 1.5 mM at t=35 days while the no-SA OSRM had a concentration of 4.1 mM at t=0 days and 2.1 mM at t=35 days. The effect of the stearic acid was not seen in the total Fe(II) concentrations because the total Fe(II) concentrations were lower at t=35 days with the SAORM.

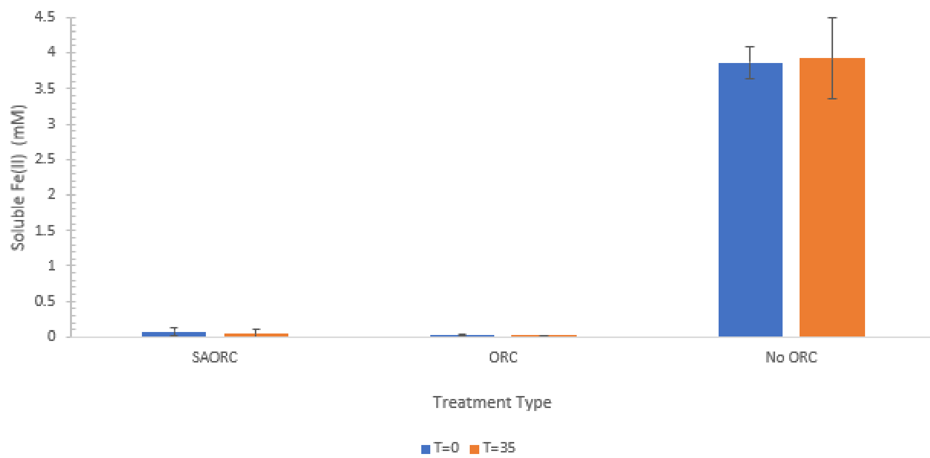

Figure 1. Soluble Fe(II) concentrations measured at t=0 days and t=35 days. Error bars display the standard deviation.

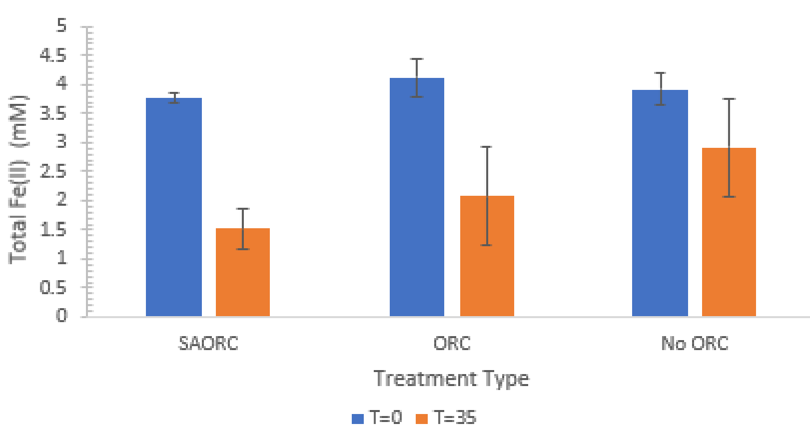

Figure 2. Total Fe(II) concentrations measured at t=0 days and t=35 days. Error bars display the standard deviation.

Discussion

This study's goal was to create OSRMs to mimic an iron snow reaction in an environment similar to Europa's ocean. Three treatments were tested: SAOSRM, no-SA OSRM, and no OSRM. These treatments were based off ORCs created in Zhou et al., (2017). CaO was used in both treatments to release oxygen and the stearic acid was used in the SAOSRM treatment to slow the rates of oxidation (Zhou et al., 2017). Both the soluble and the total Fe(II) concentrations decreased over a 35 day period, which indicated that the OSRMs prepared in this experiment did oxidize Fe(II). The SAORSM and the no-SA OSRM treatments affected the Fe(II) oxidation approximately the same. The iron snow model in this experiment imitated the reaction of the reactive oxygen species from radiolysis of the icy shell on Europa reacts with dissolved Fe(II) in the ocean to create Fe(III) oxides that will be reduced in the ocean's sediments (Lu et al., 2013). This redox reaction is important in researching Europa's ocean because it gives us a basis for the chemistry needed for a habitable environment on Europa. If this reaction does not occur on Europa, the redox gradients necessary to exchange energy from the environment for cellular energy would not be available under the hypothesized conditions of the ocean (Papalardo et al., 2009).

The oxygen releasing compounds may help future experiments concerning redox gradients in environments similar to Europa's ocean. Future research may be able to manipulate rates of oxidation and conduct experiments to examine how bacteria in environments similar to Europa's ocean respond to different rates of oxidant delivery and if these conditions would be suitable for life in Europa's ocean. This future research has exciting implications for the potential of habitability in Europa's oceans.

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